Gait Analysis in Aged Parkinsonian Mice

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Abstract

This study aimed to provide a deeper understanding of the motor impairments related to Parkinson's disease in the literature by analyzing the long-term gait performance of Parkinsonian mice possessing a unique α-syn misfolding and aggregation phenotype ($M83^{+/-}$). The aim of this thesis was to conduct a comparative analysis of gait patterns between $M83^{+/-}$ mice and a control group at 12 and 52 weeks old. This research endeavor seeked to address the gap in the current literature by providing gait analysis for hemizygous $M83^{+/-}$ mice at 52 weeks of age, as these mice have a known pathological burden at this age and are expected to exhibit gait dysfunction. The gait analysis results at 52 weeks of age show an increase in the average limb stride length variability and average limb ataxia coefficient of $M83^{+/-}$ mice, indicating gait dysfunction. However, no significant differences in several other gait parameters, such as stride length and frequency, were observed at 52 weeks between the two groups. These results are consistent with human PD progression, in which pathological symptoms precede motor decline. These hemizygous mice show slow motor impairment and disease progression, thus making them suboptimal options for studying gait in PD.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting roughly 2-3% of the population >65 years of age worldwide.[1] It is the most common neurodegenerative disease, second to Alzheimer's disease. Currently, there is no cure for PD, and the available treatments focus on alleviating symptoms rather than addressing the underlying cause. The exact etiology of PD remains unknown, but a combination of genetic and environmental factors is thought to contribute to disease development and progression.[1] PD pathology is characterized by a progressive loss of dopamine-producing neurons in the substantia nigra and an aggregation of Lewy bodies consisting of misfolded alpha-synuclein (α-syn) proteins.[2,3] These pathological symptoms correlate with motor issues such as tremors, rigidity, and bradykinesia.[1] These motor issues also contribute to the development of shuffling gait, dragging feet, and reduced arm movement that interferes with activities of daily living.[4] In addition, PD is also associated with non-motor symptoms, including gastrointestinal dysfunction, cognitive impairment, depression, and sleep disorders.[1–3] The loss of dopaminergic neurons in the substantia nigra in the midbrain is responsible for the motor impairments associated with PD.[3] A decrease in dopamine levels disrupts the communication between the substantia nigra and basal ganglia, which is crucial in motor coordination.[3]

The accumulation of Lewy bodies can occur before the onset of diagnosable motor impairment symptoms.[2] The categorization of PD among a group of neurodegenerative diseases called synucleinopathies stems from the distinctive misfolding pattern of α-syn proteins that form the bulk of Lewy bodies.[5] Additional synucleinopathies that are recognized include the Lewy body variant of Alzheimer's disease, diffuse Lewy body disease, multiple system atrophy, and dementia with Lewy bodies.[5] Despite the limited understanding of α-syn's normal function in

the brain and its role in PD, the scientific community largely acknowledges that the pathological misfolding of α-syn and the ensuing Lewy body formation are key pathological hallmarks of neurodegeneration.[6]

Several studies have utilized murine models to gain insight into the disease mechanisms of PD. Murine models are typically established by inducing Parkinsonian symptoms either through the administration of toxins or genetic manipulation.[7] However, it should be noted that murine models are inherently imperfect and cannot entirely mimic all the neuropathological or behavioral features of human PD.[7] Despite this limitation, murine models remain valuable tools for replicating a few specific pathological hallmarks of PD, such as the α-syn protein misfolding.

The regions of chromosomes or specific locations on chromosomes that are associated with PD in humans are designated by the PARK nomenclature, which comprises 18 chromosomal locations spanning from PARK 1 to PARK 18.[8] Autosomal-dominant forms of PD are linked to mutations in PARK1, PARK4, and PARK8.[8] In contrast, autosomal recessive forms are associated with mutations in PARK2, PARK6, PARK7, and PARK9.[8] This study focuses on the hemizygous $M83^{+/-}$ mouse strain, which exhibits an autosomal-dominant mutation in the Synuclein Alpha (SNCA) gene's PARK1=4 region. This gene encodes the α-syn protein and has three relevant missense mutations.[8] This study focused on the A53T missense mutation in the *SNCA* gene. Threonine is substituted for Alanine at position 53 in the amino acid sequence, causing α-syn's secondary structure to misfold from α-helices to β-pleated sheets.[8] This misfolding of α-syn's secondary structure has been linked to the manifestation of Parkinsonian symptoms, such as tremors, bradykinesia, and memory loss.[6] Since disease progression in these transgenic mice is slow, with

Although comparisons between quadruped and biped gait are known to be challenging,[9] PD gait impairment in both mice and humans show many similarities. In a previous study, M83 mice with the A53T mutation developed Parkinsonian symptoms similar to those observed in humans.[10] These symptoms included weight loss, gait abnormalities, balance/coordination deficits, and spatial memory deficits. The gait abnormalities were particularly notable, as they included a reduced stride length characteristic of Parkinson's patients with a shuffling gait.[10] Due to these similarities with human Parkinsonian presentations, A53T mice are valuable candidates for PD research.

The objective of this thesis was to conduct a comparative analysis of gait patterns exhibited by hemizygous M83$^{+/-}$ mice in contrast to those observed in a control non-disease group, which comprises the widely used C57BL/6J inbred strain. The aim was to identify any differences in the gait of these two cohorts at both the 12-week and 52-week time points, thereby providing a deeper understanding of the motor impairments related to PD. This research endeavor aimed to bridge the existing gap in the literature by providing gait analysis for hemizygous M83$^{+/-}$ at 52 weeks of age. M83$^{+/-}$ mice have pathological burden at 52 weeks[11] and should theoretically also experience gait disfunction. Due to the young age of the mice and insufficient time for disease progression and pathology to ensue, a lack of statistically significant differences in gait at 12 weeks was anticipated.

Materials and Methods

Animals

The handling and procedures of all mice were done according to the National Institutes of Health Guide for the Care and Use of Experimental Animals. They were approved by the University of Florida Institutional Animal Care and Use Committee. A cohort of 14 mice was

used to complete this study, 6 of which belonged to the hemizygous M83 transgenic mouse strain ($M83^{+/-}$) that exhibited the A53T missense mutation on the SCNA gene in the PARK1=4 loci. The remaining 8 mice were from the C57BL/6J inbred strain and were used as control animals.

The Digigait

This study utilized the DigiGait system, an integrated treadmill and imaging apparatus manufactured by Mouse Specifics, Inc., to record and analyze the gait of the mice.[12] The system is designed with a transparent polycarbonate compartment that limits the animal's range of movement to a specific treadmill area. The dimensions of the compartment were adjusted to accommodate an average-sized mouse, measuring 6.5" x 2" x 6". The treadmill's speed was maintained at a constant 20 cm/s throughout the study, despite using slower speeds to acclimate the mice before recording. This was selected to ensure a consistent speed that all mice could achieve throughout the experimental timeline, including the sicker mice toward the end of the study. Previous studies involving mice of the same strain also reported using a 20 cm/s speed.[10,13] The treadmill could be stopped by setting the walking speed controls to 0.00 cm/s or using the on/off button.

Upon recording a video of the mouse's movement on the treadmill, the DigiGait software of Mouse Specifics Inc. was utilized to quantify various qualitative aspects of the gait.[12] The software assigns specific numerical values and ranges to parameters such as stride width and paw angles. Once the paw detection process is complete, the user can edit the graphical data outputs of the gait signals, enabling the removal of compression artifacts or errors that may have occurred during the analysis. Artifacts may arise if the software registers anything other than the paws. Moreover, errors may result if the mouse touches the compartment's sides, jumps, stops, or varies speed during the recording. Therefore, the mouse must maintain continuous movement at

a uniform rate, equidistant from the boundaries of the polycarbonate compartment. After editing, the software considers the video footage fully processed. The videos were saved and exported for further organization by third-party applications with graphical and statistical capabilities.

Indices

The DigiGait system offers many available indices with different numerical values that apply to each paw for each recording (Figure 1).[12] The fundamental indices analyzed in this thesis include stride time, stride length, stride frequency, stride length variability, swing, stance, and ataxia coefficient.

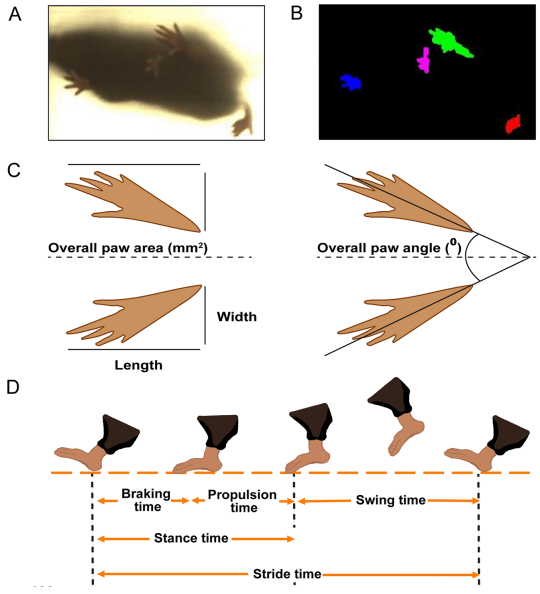

Figure 1: *Depiction of Digigait imaging system and gait parameters. (A) Ventral image of a mouse on the Digigait treadmill. (B) The digital heatmap of the ventral paw prints. (C) Diagram showing how paw prints are used to measure certain gait parameters. (D) Illustration of temporal gait parameters.*[14]

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Stride time describes the time taken to complete a full step and can be calculated by the sum of stance and swing durations. Stride length refers to the distance traveled in cm by a single paw during a stride. Stride frequency, in turn, refers to the frequency or number of times a paw completes a stride in a second. Stride length variability is a measure of the constantly changing

stride lengths throughout a run. Swing refers to a suspended paw's duration without contact with the treadmill track during a step. Stance is the duration during which the paw is in contact with the treadmill track. The ataxia coefficient is a measure of step variability that increases in PD.

Data collection

Gait was evaluated using the DigiGait at two time points: 12 weeks and 52 weeks to assess any gait alterations between groups that were at baseline compared to when they were allowed to age, but before they exhibited any overt Parkinsonian symptoms. Mice were first trained on the DigiGait apparatus by running on the treadmill at a speed range of 15 to 20 cm/s for approximately 30 seconds. After this, the mice were allowed to rest for at least 1 hour. Subsequently, each mouse was run on the treadmill at a constant speed of 20 cm/s with no incline until four to five seconds of reliable data was obtained. Reliable data was defined as a recording depicting a mouse running with a uniform speed and gait equidistant from the boundaries of the polycarbonate compartment. Any disruptions such as a mouse running too close to the front or back of the compartment, paw contact with the sides of the compartment, jumping or abrupt stoppage necessitated re-recording. The recording duration of four seconds was chosen to achieve an average of 15 uninterrupted sequential steps per recording. The treadmill was cleaned with 70% ethyl rubbing alcohol between each mouse run to eliminate any residual urine or fecal matter that could impact the subsequent mouse's gait performance. The gait for all 14 mice was then evaluated again when they reached 52-53 weeks old. The collected data was then analyzed utilizing the DigiGait software.

Statistical Analysis

Data was organized on Microsoft Excel and analyzed multiple times using Excel and PRISM, which conducted multiple two sample t-tests assuming unequal variances.

Results

At 12 weeks of age, there were significant differences in average hind and average limb swing time, which were unexpected (Figure 2). M83$^{+/-}$ group showed increased swing time in hind limbs and when taking the average limb swing time. All other indices compared between the groups were not significant in mice at 12-13 weeks.

For mice aged 52 weeks, there were no statistically significant differences detected in the parameters of stride length or stride frequency. Similarly, differences in stance time, stride time, and swing time were not significant. However, the M83$^{+/-}$ group did show significant differences from the C57 control group regarding average limb stride length variability (Figure 3A) and the average limb ataxia coefficient (Figure 3B). The average limb variability in stride length and ataxia coefficient were both increased in diseased mice indicating an increase in gait instability compared to the control group.

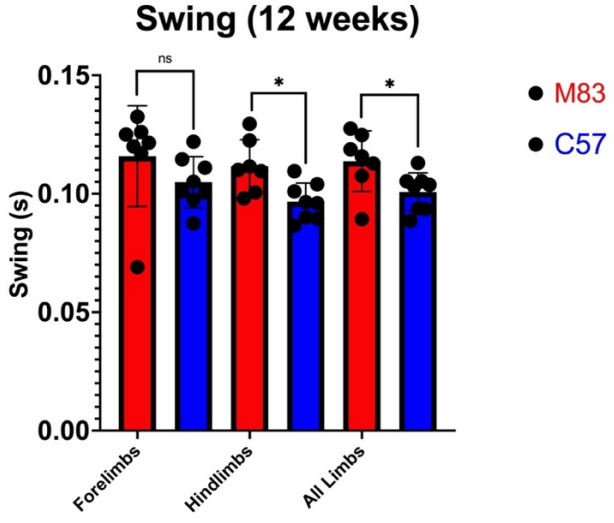

Figure 2: *Mean values shown for swing time (s) from Digigait analysis between M83$^{+/-}$ and C57 control for mice aged 12 weeks. Means were analyzed separately for forelimbs, hindlimbs, and all limbs. Analysis shows significant difference at $\alpha = 0.05$ between the average swing time of the hindlimbs with $p = 0.0135$. Analysis shows significant difference at $\alpha = 0.05$ between the average swing time of all limbs combined with $p = 0.0432$.*

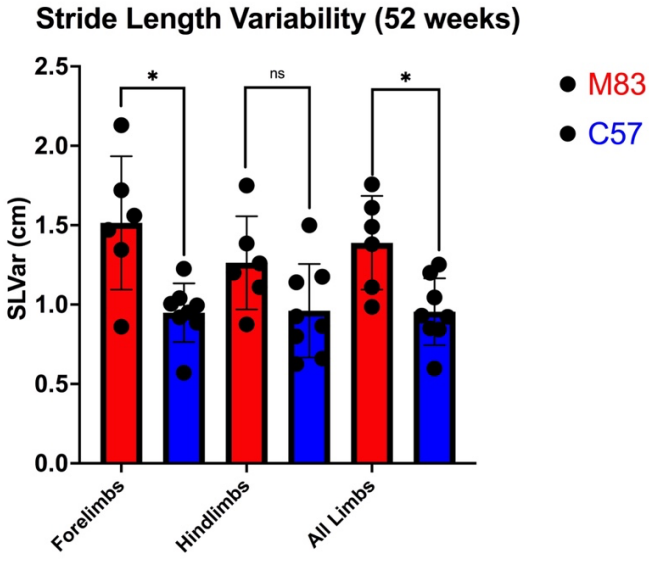

Figure 3A: *Mean values shown for stride length variability (cm) from Digigait analysis between M83$^{+/-}$ and C57 control for mice aged 52 weeks. Analysis shows significant difference at $\alpha = 0.05$ between the average variability in stride length of the forelimbs with $p = 0.0197$. Analysis shows significant difference at $\alpha=0.05$ between the average variability in stride length of all limbs combined with $p = 0.0142$.*

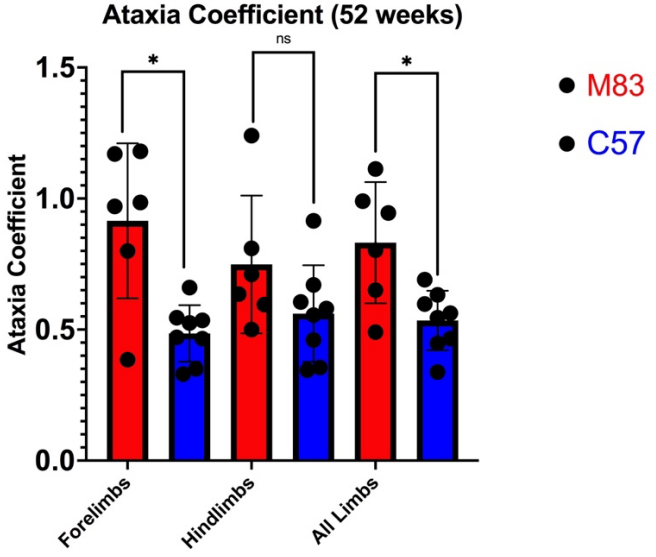

Figure 3B: *Mean values shown for ataxia coefficient (real number) from Digigait analysis between M83$^{+/-}$ and C57 control for mice aged 52 weeks. Analysis shows significant difference at $\alpha = 0.05$ between the average variability in stride length of the forelimbs with $p = 0.0145$. Analysis shows significant difference at $\alpha = 0.05$ between the average variability in stride length of the forelimbs with $p = 0.0238$.*

Discussion

Gait metrics were not expected to be significantly different at 12 weeks between M83 and C57 mice. Nevertheless, the $M83^{+/-}$ mice demonstrated a statistically significant increase average limb and hindlimb swing time. A high p-value of 0.0432 for the mean limb swing time, coupled with the absence of any other statistically significant gait metrics, suggest the existence of extraneous variables that impacted the measured outcome of swing time.

It was hypothesized that the aged $M83^{+/-}$ group, which has a mutation in the *SCNA* gene resulting in misfolding of α-syn proteins and subsequent motor issues, would exhibit significant differences in gait at 52 weeks compared to the aged control group, C57. Despite previous studies emphasizing the significance of stride length and stride frequency for distinguishing between diseased and control mice in the homozygous setting,[10,15] the results did not identify any variation in those measures in the heterozygous setting, without IM PFFs. Stride lengths were found to be decreased in both hemizygous $M83^{+/-}$ mice injected with α-syn fibrils[15] and 12 month old homozygous $M83^{+/+}$ mice[10]. The data for stride lengths in this study did not align with these previous findings, potentially due to the supplemental α-syn fibril injection and delayed onset of disease progression and neurological symptoms for hemizygous $M83^{+/-}$ mice, which typically occurs around 24 months of age.[11] Interestingly, inconsistencies in stride frequencies have been noted in the literature, with one study showing decreased frequencies in homozygous mice[10] and another showing increased frequencies in hemizygous mice with α-syn fibril injection.[15] The increased stride frequency relates better to PD in humans, as patients often display festinating gait, which is defined by short, frequent steps, trunk leaning, and balance control deficits that are all compensatory mechanisms for decreased stride length.[16]

The outcomes of the study demonstrated notable differences in both the ataxia coefficient and stride length variability. An increase in stride length variability is consistent with murine models and clinical findings that have also reported an increase in variability.[17,18] One potential avenue for future gait studies is to incorporate the variability in stride length as a key parameter, as it further reinforces the association between the motor symptoms of PD in mice and humans. An increase in ataxia coefficient was anticipated and aligns with prior results from PD mice gait analyses.[19] Despite finding significant differences in both stride length variability and ataxia coefficient that support the hypothesis, using hemizygous $M83^{+/-}$ mice may not be the most optimal option for PD research. There were no significant differences found in several fundamental gait measures between the groups. These hemizygous mice have slow disease progression, making them less suitable for studying PD. Instead, using hemizygous mice that have been injected with α-syn fibrils or homozygous $M83^{+/+}$ may provide better results when researching treatments to alleviate PD symptoms in mice.

This study had a few limitations. Although the initial hypothesis predicted no differences between the experimental groups at 12 weeks and significant gait differences at 52 weeks, the results did not support these expectations. Several limitations, such as the sample size insufficiency, may have contributed to these outcomes. Specifically, the limited sample size may have influenced the measured increase in $M83^{+/-}$ swing time at 12 weeks, which could have been affected by outliers skewing the results. The study's limited sample size could have also influenced the lack of significant differences found in several Digigait indices at 52 weeks. A larger starting sample size would provide more precise results by accounting for mice that do not survive until the 52-week time point. One plausible explanation for the lack of significant gait differences observed at the 52-week time point is that one year may not have been sufficient for

all the M83$^{+/-}$ mice to experience genuine gait disturbances. While disease pathology is known to occur in these mice at age one-year[11], significant gait disturbances and actual PD diagnosis in humans typically occur after disease pathology.[20] Furthermore, the use of outdated Digigait fortware presented another limitation of the study. The transparency of the treadmill track had degraded over time, thereby making it more challenging for the software to discern the paws of the mouse. Consequently, the automated analysis may have failed to detect the full paw of the animal, leading to a higher probability of error in the subsequent gait measurements.

Conclusion

This study performed a long-term comparative analysis of the gait of hemizygous M83$^{+/-}$ mice to the inbred control group, C57. The goal was to find significant differences in several measurements of gait when the mice were 52 weeks old. Surprisingly the diseased group showed significantly increased stride time at 12 weeks. This may have been due to extraneous variables, as no other gait parameter showed significant differences between groups. Stride length and frequency, two gait parameters significantly affected in both mice and human PD, showed no significant difference between the groups. This study did find significant differences in average stride length variability and average ataxia coefficient at 52 weeks of age. The average limb stride length variability was increased in the diseased mice, which correlates with human PD. The ataxia coefficient was significantly higher in the M83$^{+/-}$ group, which supports the initial hypothesis. However, the overall gait of hemizygous M83$^{+/-}$ was not significantly different from C57 control at 52 weeks. Thus, future studies regarding PD mice should utilize homozygous M83$^{+/+}$ or inject supplemental α-syn fibrils into M83$^{+/-}$. Studies using M83$^{+/-}$ mice would take too long without peripheral α-syn fibril injection. Future studies could assess the gait of M83$^{+/-}$ mice

at 104 weeks to ensure that the pathological PD symptoms present in these mice at 20-26 months,[11] is consistent with abnormal gait.

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